Obscn Cas9-CKO Strategy

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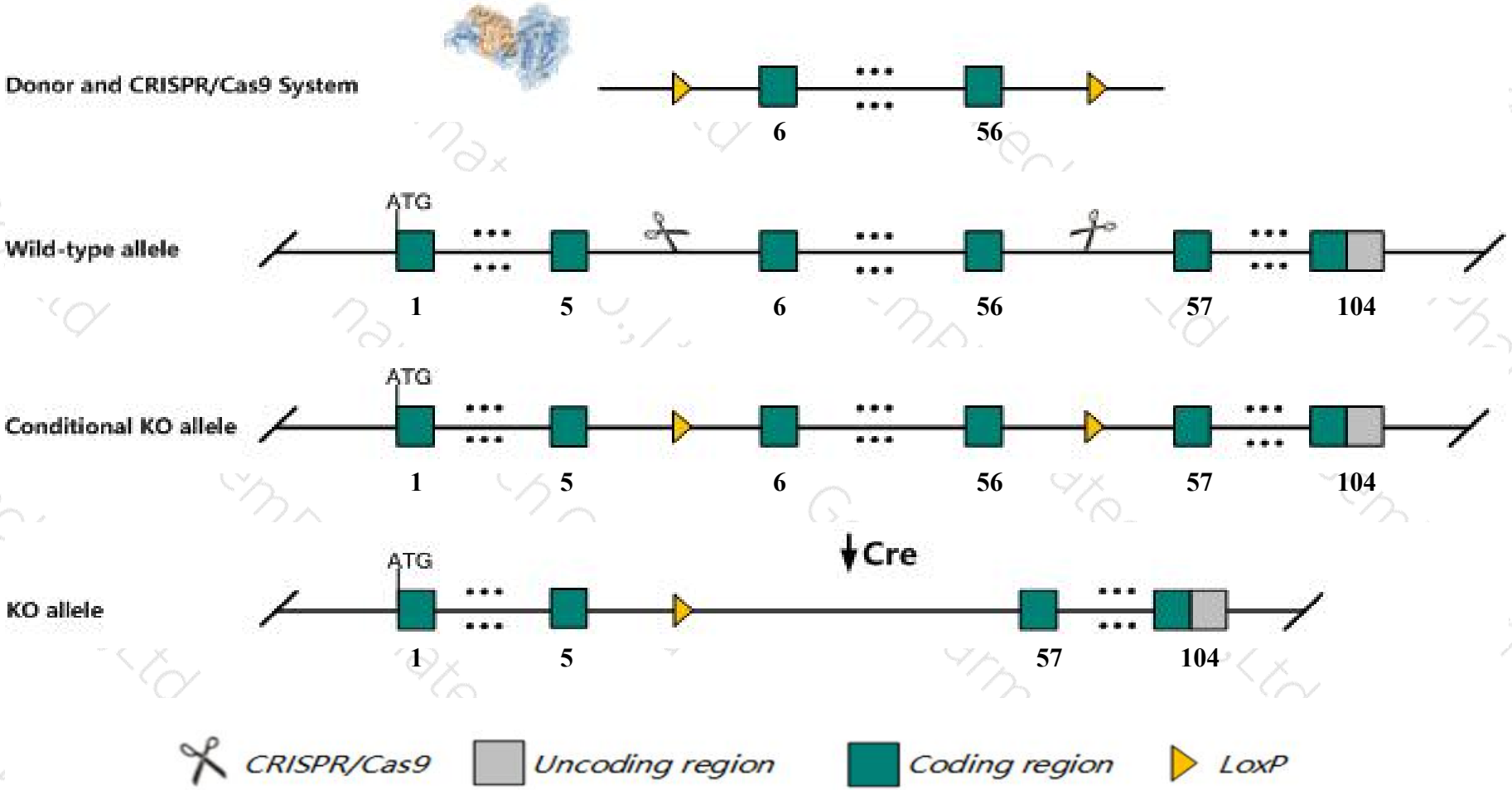
Project Overview

Project Name	***Obscn***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Obscn* gene. The schematic diagram is as follows:



Technical routes

- The *Obscn* gene has 10 transcripts. According to the structure of *Obscn* gene, exon6-exon56 of *Obscn-202* (ENSMUST00000047441.13) transcript is recommended as the knockout region.The region contains 13100bp coding sequence Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Obscn* gene. The brief process is as follows:CRISPR/Cas9 system and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice.Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

- The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

Notice

➢ According to the existing MGI data,Mice homozygous for a knock-out allele exhibit centrally localized nuclei in muscle fibers and mild myopathy in aged mice.

➢Some amino acids will remain at the N-terminus and some functions may be retained.

➢Transcript 205,208 CDS 5' and 3' incomplete the influences is unknown.

➢ The *Obscn* gene is located on the Chr11. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.

➢ This Strategy is designed based on genetic information in existing databases.Due to the complexity of biological processes, all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

集萃药康 GemPharmatech

Obscn obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF [Mus musculus (house mouse)]

Gene ID: 380698, updated on 19-Mar-2019

Summary

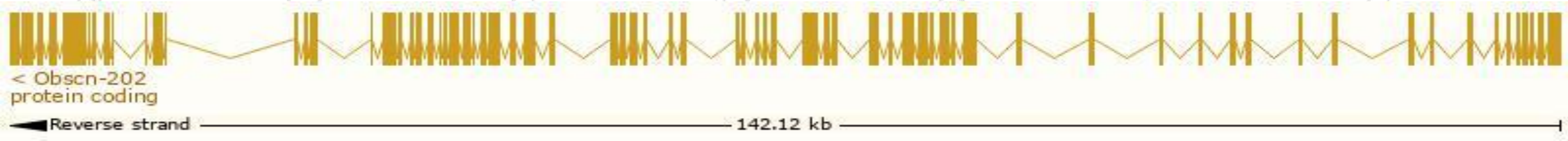
Official Symbol	Obscn provided by MGI
Official Full Name	obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF provided by MGI
Primary source	MGI:MGI:2681862
See related	Ensembl:ENSMUSG00000061462
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	BC046431, Gm878, UNC89
Summary	The obscurin gene spans more than 150 kb, contains over 80 exons and encodes a protein of approximately 800 kDa. The encoded protein contains 68 Ig domains, 2 fibronectin domains, 1 calcium/calmodulin-binding domain, 1 RhoGEF domain with an associated PH domain, and 2 serine-threonine kinase domains. This protein is one of three giant sacromeric signaling proteins that includes titin and nebulin. It may have a role in the organization of myofibrils during assembly and also may mediate interactions between the sarcoplasmic reticulum and myofibrils. Alternatively spliced transcript variants encoding different isoforms have been described although the full-length nature is not known for all splicing variants. [provided by RefSeq, Jan 2010]
Expression	Biased expression in heart adult (RPKM 40.7), mammary gland adult (RPKM 7.2) and 1 other tissue See more
Orthologs	human all

Transcript information（Ensembl）

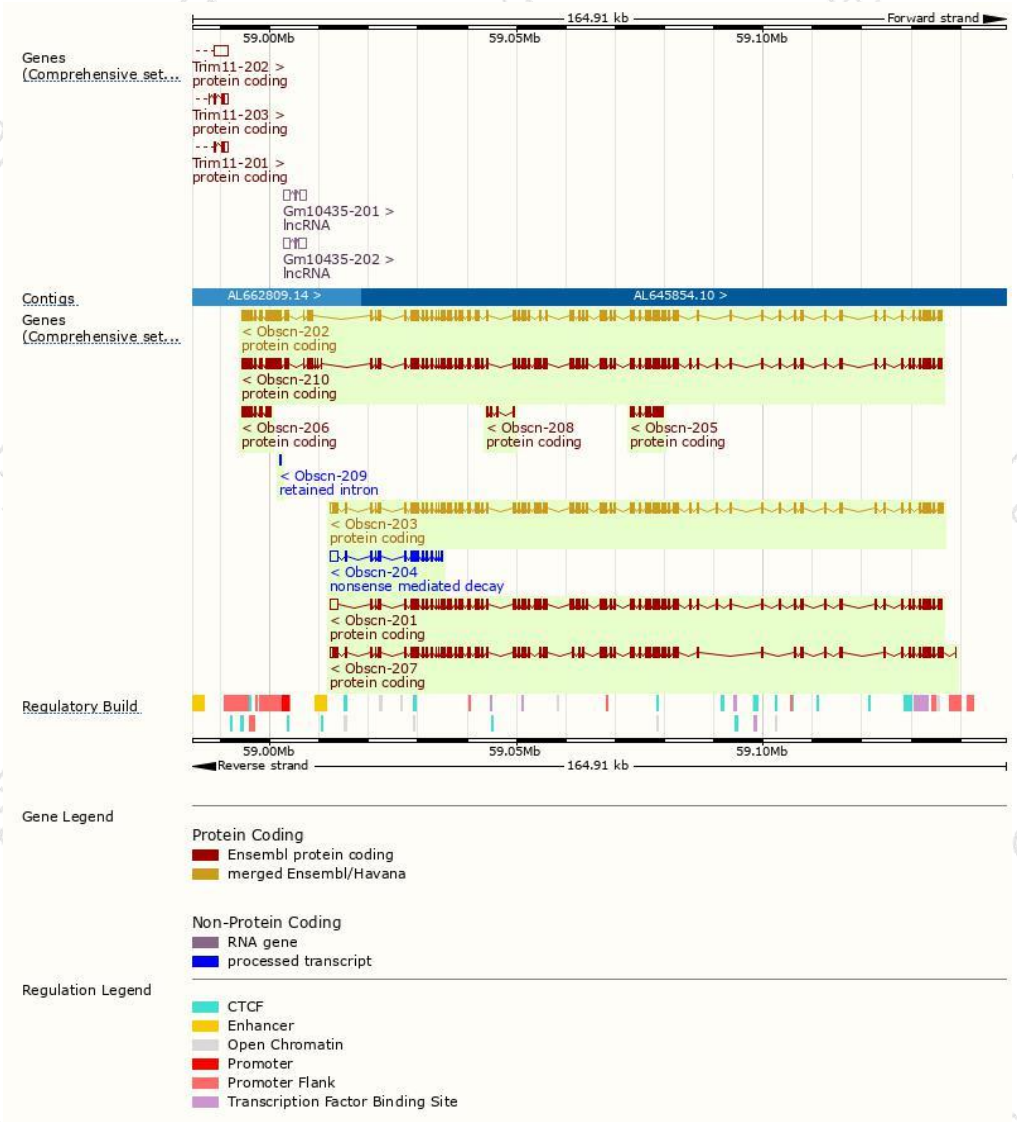
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Obscn-202	ENSMUST00000047441.13	24175	8032aa	Protein coding	CCDS56775	H7BX05	TSL:5 GENCODE basic
Obscn-203	ENSMUST00000052872.14	23027	7496aa	Protein coding	CCDS56774	E9QQ96	TSL:5 GENCODE basic
Obscn-210	ENSMUST00000238536.1	26737	8886aa	Protein coding	-	-	GENCODE basic APPRIS P1
Obscn-201	ENSMUST00000020732.12	23163	7176aa	Protein coding	-	Z4YJE4	TSL:5 GENCODE basic
Obscn-207	ENSMUST00000219084.2	20567	6661aa	Protein coding	-	A0A1W2P6H1	TSL:5 GENCODE basic
Obscn-206	ENSMUST00000138587.8	2848	923aa	Protein coding	-	F6TJX7	CDS 5' incomplete TSL:5
Obscn-205	ENSMUST00000133040.3	2548	849aa	Protein coding	-	J9JIB2	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Obscn-208	ENSMUST00000238202.1	889	296aa	Protein coding	-	-	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete
Obscn-204	ENSMUST00000132238.7	4154	221aa	Nonsense mediated decay	-	F7DCJ0	CDS 5' incomplete TSL:5
Obscn-209	ENSMUST00000238245.1	356	No protein	Retained intron	-	-	

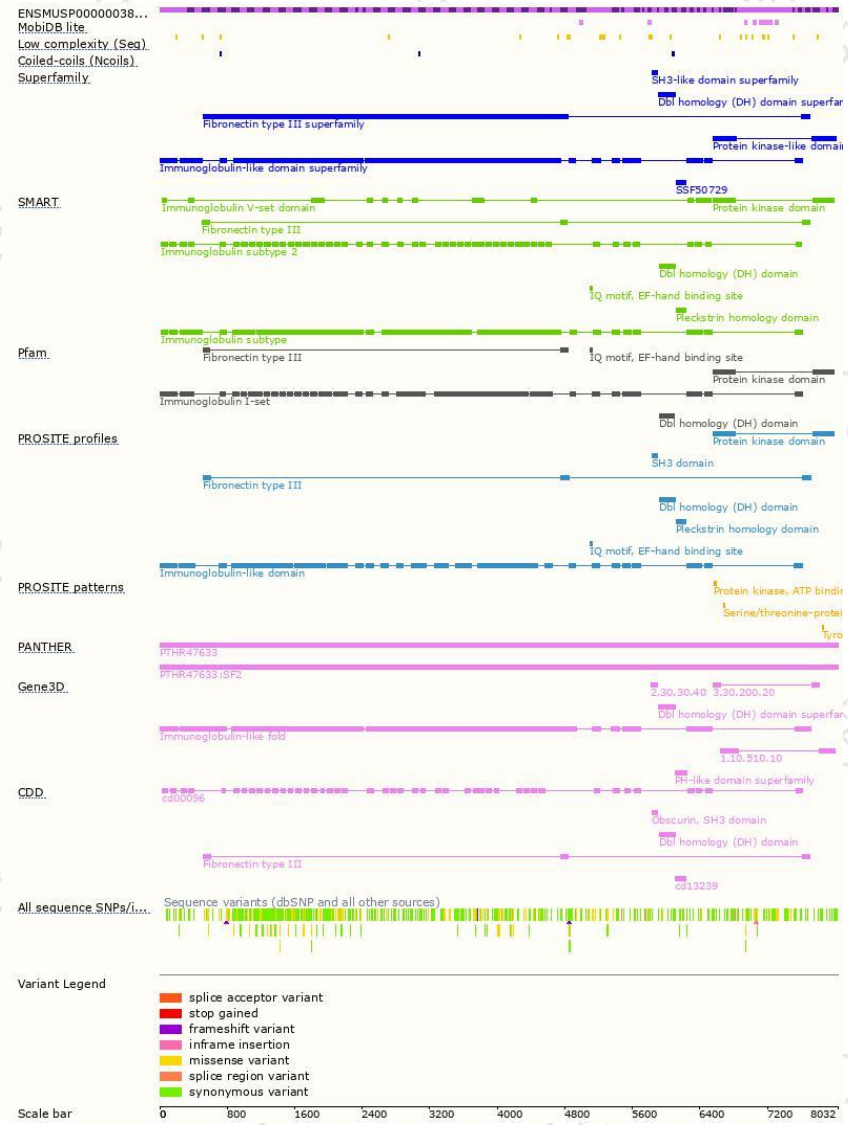
The strategy is based on the design of *Obscn-202* transcript,The transcription is shown below



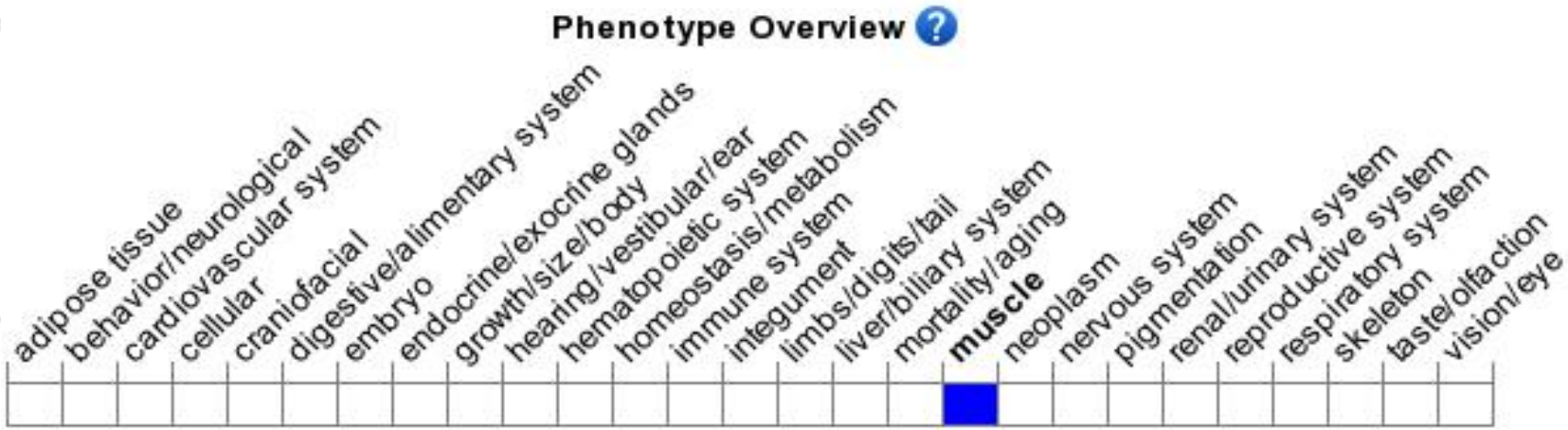
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

According to the existing MGI data,Mice homozygous for a knock-out allele exhibit centrally localized nuclei in muscle fibers and mild myopathy in aged mice.

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If you have any questions, you are welcome to inquire.

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